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APPLICATION OF CALCULATED SEDIMENTATION RATIOS IN THE SPECIFICATION OF MODELS FOR PROTEIN DIMERS, TRIMERS, TETRAMERS AND PENTAMERS

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In the preceding paper a systematic method for delineating quaternary structures compatible with the sedimentation behaviour of oligomeric assemblies was developed. In this companion paper the approach is illustrated by its application to a number of oligomeric proteins, namely β-lactoglobulin, aminoacyl transferase, succinic semialdehyde dehydrogenase, arginine decarboxylase, and the arthropod hemocyanins. Structures of these proteins based on comparisons of calculated and experimental sedimentation coefficients are presented and, wherever possible, compared with those obtained by other techniques. The usefulness and limitations of the method are assessed. It is concluded that the sedimentation analysis will usually yield a reasonably good representation of the mode of assembly of protein molecules in oligomeric structures if accurate experimental data are available. A high degree of resolution of structural detail is not expected and since, in addition, the sedimentation ratio method is seldom definitive to one structure, it is most valuable when used in a conformatory or eliminative way with results from techniques more specifically designed for structure determination.

1. Introduction

In the preceding paper [1] and in [2] we presented calculated sedimentation ratios and dimensions for oligomers in which up to six identical protomers are assembled in spatially equivalent positions. The range of protomer shapes employed was especially relevant to oligomeric proteins, as was the number of protomers considered, although the results can of course be applied to the interpretation of sedimentation velocity results obtained with suitable aggregates of any kind of subunit. In the present work we apply the suggested approach to data obtained with actual oligomeric proteins to illustrate its use and assess its limitations.

It was hope that it would be possible to deduce a structure, or a limited number of alternative structures, for an oligomer of each type on the basis of sedimentation data alone, and then to compare these with structures known with a fair degree of certainty from other techniques. This aim requires that a rather extensive set of data be available for each protein considered; as will be seen, this was not strictly possible for every kind of oligomer. Instead, we consider in turn a number of oligomeric proteins for which most of the required data are available. Sufficient examples were chosen that all of the oligomers, up to and including pentamers, are represented, usually more than once. Whenever possible, structures deduced on the basis of the sedimentation ratio method are compared with those from other techniques, such as electron microscopy or X-ray crystallography. In some cases such comparisons are not possible; the results of the sedimentation ratio analysis are then used as a basis for comment on possible solution structures of the oligomers in question.

2. Examples

2.1. β-lactoglobulin

2.1.1. Dimer formation

There is a very extensive literature on the polymerization behaviour of β-lactoglobulin and its genetic variants under a variety of conditions; there is also some ambiguity in the terminology. In this section the

aggregation of two molecules of molecular weight 18000 to form the dimer, of molecular weight 36000, is considered. The value for $S^{\circ}_{20,w}$ for the monomer is given as 1.90 S by Townend et al. [3] who assesses the error in their low concentration data as about 5%. In view of this, and of the steep extrapolation to zero concentration involved, a realistic range of values for the sedimentation coefficient of the monomer ($S^{\circ}_{20,w}$) is 1.90–2.0 S.

The partial specific volume is taken as 0.75 ml/g [4] and the hydration to be in the range 0.3 g/g to 0.5 g/g. The lower limit corresponds to the measured "unfreezable water" [5] and the upper to that calculated from the hydration of the constituent amino acids [4]. From the molecular weight and partial specific volume a value of f/f_0 of 1.18 can be calculated for a sedimentation coefficient of 1.9 S and an f/f_0 of 1.12 for a sedimentation coefficient of 2.0 S. These two values in conjunction with the hydration data and the diagram of axial ratios for ellipsoids of revolution given by Oncley [6] yield the possibilities for protomer shape given in table 1.

The value of $S^{\circ}_{20,w}$ for the β-lactoglobulin dimer is given by Townend at al. [3] as 3.04 S and by Lee and Timasheff [4] as 2.87 S. The experimental range of values for the sedimentation ratio is therefore taken to be 2.87/2.0–3.04/1.90 i.e. from 1.44 to 1.60. Figs. 3 and 4 in the preceding paper [1] may now be consulted to ascertain the dimeric structures which are compatible with the quoted ranges of protomer shape and sedimentation ratio. It is evident that quite a large number of structures may be accommodated within these values, the extremes in terms of protomer shape being $Hr180O_2$, Hr0S and $Hr0P_2$ as illustrated in fig. 1. Only the first two satisfy the conclusion of a crysta lographic study [7] that an axial ratio of 2 : 1 and a two-fold axis of symmetry at right angles to the length appear obligatory for the dimer. The sedimentation analysis alone cannot yield more definite results in this case but it is in agreement with the assessment from the X-ray analysis that a structure consisting of two slightly flattened spheres in contact is the best model. The sedimentation analysis by Townend et al. [3] led them to the same conclusion, and indeed the present treatment adds nothing new to the picture of the β-lactoglobulin dimer. It illustrates that in general more than one structure may be compatible with sedimentation velocity data when realistic account is taken of the possible deviation of protomer shape from spherical and of experimental error. Then it will be necessary to combine the results from different techniques in order to eliminate some possibilities.

Table 1
Possible shapes for β-lactoglobulin monomer

f/f_0	Hydration (g/g)	Protomer shape
1.12	0.3	Prolate or oblate 1.3 : 1
1.12	0.5	Spherical
1.18	0.3	Prolate or oblate 2 : 1
1.18	0.5	Spherical

The upper and lower limits of frictional ratios and hydrations are experimental determinations and the combinations shown lead to the indicated different possibilities for the shape of the protomer.

2.1.2. Octamer formation

The reaction referred to is actually an example of a tetramerization exhibited by β-lactoglobulin A at acid pH [8] in which four molecules of the dimer of molecular weight 36000 aggregate to an octamer of molecular weight 144000. The protomer is thus the dimer, which, as we have seen, is best described as two slightly flattened spheres in contact, i.e. P_2 in the present terminology.

The sedimentation coefficient for the octamer has not been independently measured since the dimer and octamer are in rapid equilibrium. However, Gilbert [9] obtained an excellent fit to the weight average sedimentation coefficient versus concentration data for this reaction by taking the sedimentation coefficients ($S^{\circ}_{20,w}$) to be 2.87 S for the dimer and 7.37 S for the octamer. The sedimentation ratio for the tetramerization is then 2.57. Reference of fig. 6 of [1] shows that for this value of the sedimentation ratio and a protomer shape P_2 the only possible structure is that denoted CVV and illustrated for this particular case in fig. 1. Gilbert [9] has such a compact structure in mind in assigning the sedimentation coefficient of the octamer and it is satisfying that the present approach verifies the appropriateness of such a model. The structure resulting from the sedimentation analysis is essentially a cube of side about 70Å (table

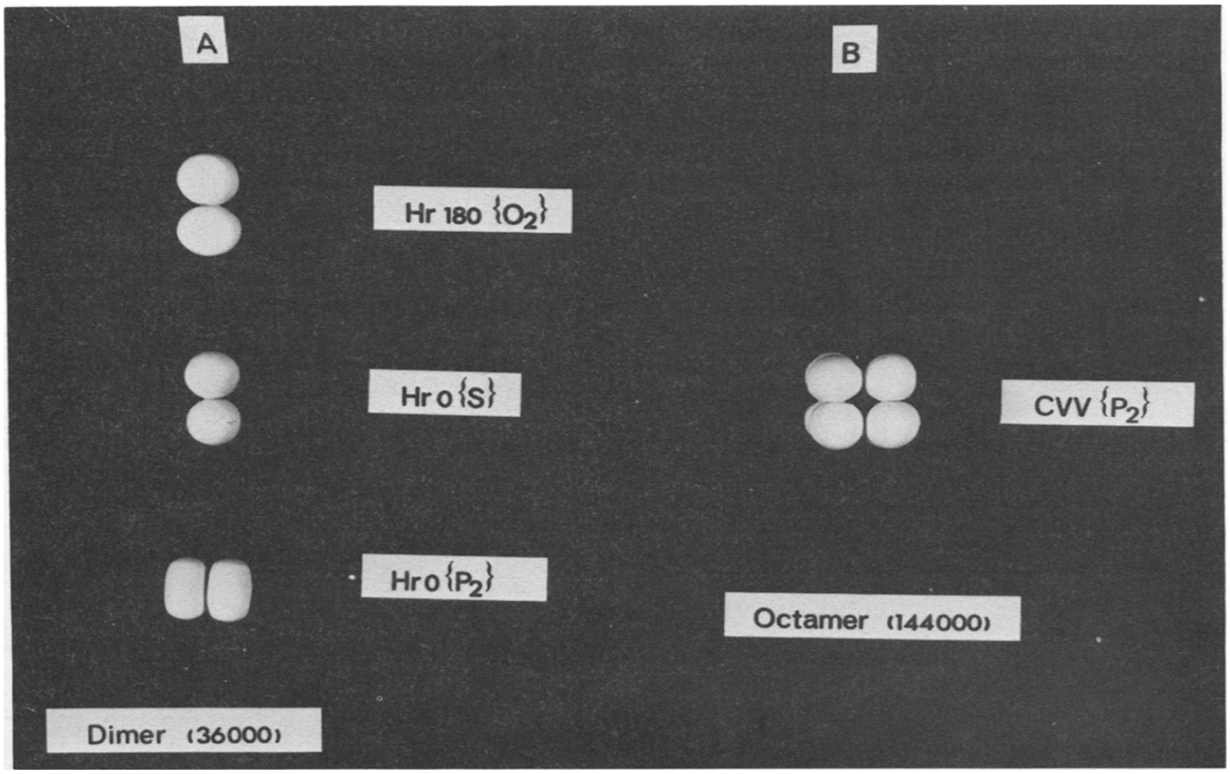

Fig. 1. Structures for β-lactoglobulin dimer and octamer. (A). The monomeric protomers in the dimeric models are all of the same volume, the shapes being selected on the basis of sedimentation data as described in the text and table 1. Oblate and prolate protomers are shown as rounded discs or cylinders, respectively, of the appropriate axial ratio. These are effectively the shapes to which the method of calculation refers. The labels denote the mode of assembly and protomer type as described in [1]. (B). The octamer resulting from association of four dimeric protomers, each effectively P_2 being formed from two slightly flattened spherical monomers.

4 of [1]). The shape and dimensions are in good agreement with the conclusion reached from recent measurement of the diffusion constant of the β-lactoglobulin octamer that the molecules behaved like close-packed spheres with an effective radius of 45 Å [10].

2.2. *Two protein trimers*

There are a number of well authenticated trimeric proteins including three (bacteriochlorophyll protein [11]. glucagon [12], and 2-keto-3-deoxy-6-phosphogluconate aldolase (KDPGA) [13]) for which detailed structures are available. Unfortunately for the present purpose there is no example in which sedimentation coefficients of both monomer and trimer, and a structure, are available. Often this is because it is not possible to dissociate the trimer without recourse to concentrated urea or guanidine solutions [14–16]. There are some sedimentation velocity data available for the two trimeric proteins discussed below and we use the sedimentation ratio approach here to comment on possible assembly patterns.

2.2.1. *Aminoacyl transferase*

Sedimentation equilibrium experiments showed that the molecular weight of the undissociated protein is 186000, while in 6M guanidine hydrochloride or 8M urea it is 62000 [15]. Thus it appears to be trimeric although the authors noted that the relatively low $S^{\circ}_{20,w}$ value of 6.7 S for a protein of molecular

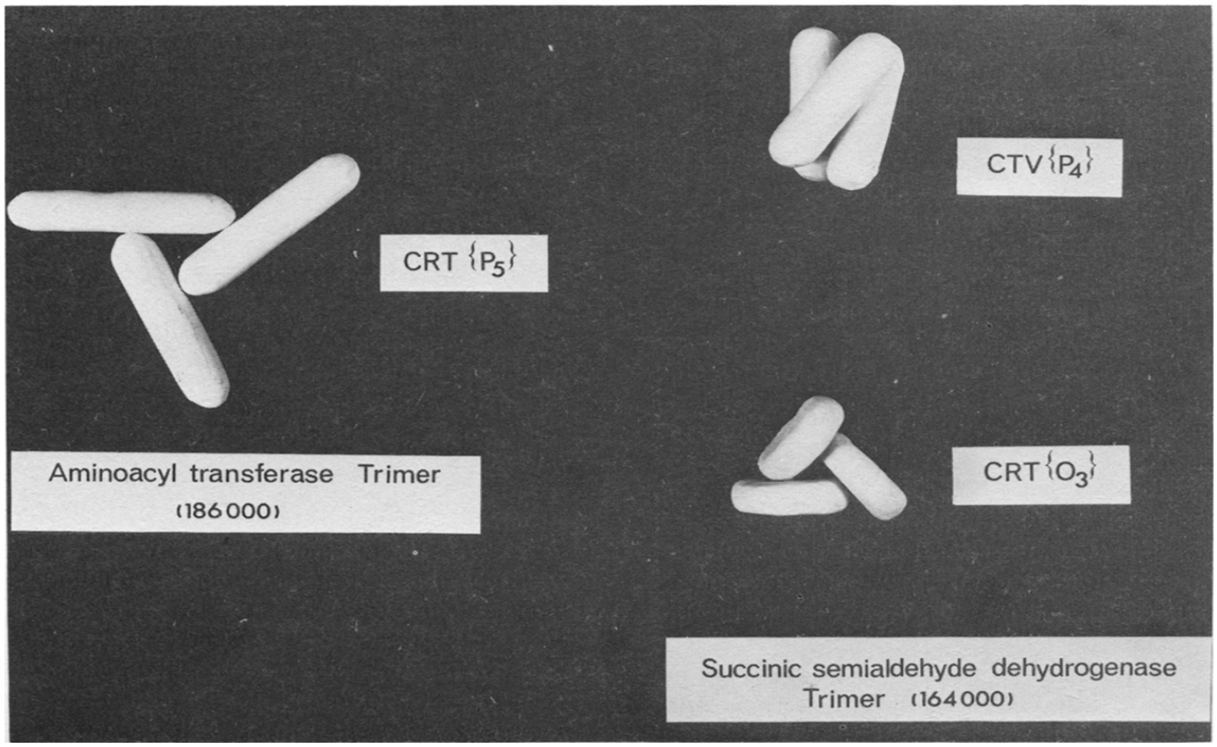

Fig. 2. Model structures for two trimeric proteins. All of the monomeric protomers in the models have been constructed with the same volume. Oblate and prolate protomers are shown as rounded discs or cylinders, respectively, of the appropriate axial ratio. These are effectively the shapes to which the method of calculation refers. The structures shown, though consistent with the sedimentation behaviour, are not definitive, see text.

weight 186000 suggests that the native enzyme may have unusual physical properties. Presumably this statement refers to the fact that a spherical molecule of molecular weight 186000 and $\bar{v} = 0.745$ ml/g (the value used by the authors [15]) would have a sedimentation coefficient of 11.0 S and consequently a frictional ratio $f/f_0 = 1.64$, a value implying an axial ratio of about 10 : 1 even for a protein with an average degree of hydration. The result illustrates that the visualization of aggregated structures simply in terms of spheres of prolate or oblate ellipsoids of revolution is likely to be misleading. It is not difficult to show, using the calculated sedimentation ratios, that the sedimentation coefficient of 6.7 S does not demand a dramatically asymmetric structure for the trimer, merely non-spherical protomers of high axial ratio in a suitable arrangement. For example, suppose an average hydration of 0.3 g/g and an axial ratio of 5 : 1 (prolate) were assumed for the monomer. With a molecular weight of 62000 and a $\bar{v}$ of 0.745 ml/g, this is equivalent to $f/f_0 = 1.40$ or a sedimentation coefficient of 3.8 S. The sedimentation ratio would then be 6.7/3.8 = 1.76 and fig. 5 of [1] shows that this is accommodated with P_5 protomers, by the structure CRT. There is nothing apparently abnormal about such a structure, which is shown in fig. 2; in fact it is very similar to the structure of the trimeric KDPGA [13] and glucagon [12].

2.2.2. Succinic semialdehyde dehydrogenase

The native protein has been shown to have a molecular weight of 164000 at neutral pH and to dissociate to three equal sized particles of molecular weight 54000 at pH 9.7 [17]. Sedimentation coefficients for both of these species have been measured and are $S^{\circ}_{20,w} = 9.45$ S and 4.0 S respectively. Although these

have not been corrected for concentration dependence the authors report that varying the protein concentration had little or no effect on the observed sedimentation coefficients [17].

From the reported error of ± 0.07 S in the sedimentation coefficient at the value of $\bar{v}$ of 0.725 [17], the frictional ratio of the monomer, f/f_0, can be calculated to be 1.33 ± 0.02. No value is available for the hydration; an average value for a protein of 0.3 g/g may be assumed, and together with the f/f_0, reveals that the monomer may be approximated as either a prolate or oblate ellipsoid of revolution of axial ratio 3–4 : 1. No error is quoted for the sedimentation coefficient of the trimer but it may be assumed to be about ±0.05 S. Thus the sedimentation ratio range is 8.40/4.07–8.50/3.93, i.e. 2.06–2.16. From the graph for the sedimentation ratios of trimeric assemblies (fig. 5 of [1]) it may be seen that the above data are consistent with two rather different structures. One is that denoted CRT with O_3 protomers, the other CTV with P_4 protomers and these are shown in fig. 2. It may be pointed out that the value of the sedimentation ratio allows elimination of all but such trimeric structures even in the absence of information about the protomer shape. Thus the assumption of a value for the hydration does not greatly affect the number of possible oligomeric structures in this instance. Even the extremes, namely P_5 protomers (corresponding to a hydration of zero) or S (corresponding to a hydration of 1g/g) do not affect the indicated mode of assembly, which may be CRT with oblate protomers or CTV-CVV with prolate protomers.

Although it seems likely that the trimeric enzyme is either a CRT structure composed of flattened spheres of axial ratio 2–3 : 1 or a CTV-CVV arrangement of elongated spheres of axial ratio up to 4 : 1 it is not possible to choose between these alternatives without more information. It could be noted that there is evidence that a related trimeric enzyme, 4 aminobutanal dehydrogenase from a *Pseudomonas* species is capable of forming hybrids with succinic semialdehyde dehydrogenase [18]. This argues for a similar structure for these two enzymes.

2.3. Arginine decarboxylase

The native molecule has a molecular weight of 820000 and dissociates readily into five apparently identical particles of molecular weight 158000 in the absence of divalent cations and at pH values above pH 6.5 [19,20]. There is evidence that no conformational change accompanies dissociation and that the protomers in the pentamer of molecular weight 820000 are actually dimeric, because a further dissociation of the 158000 units to particles of molecular weight 77000–89000, which appear to be identical, occurs in denaturing solvents [20].

Values of $S^{\circ}_{20,w}$ for protomer and pentamer are given as 8.0 S and 23.3 S, respectively [19] and examination of the experimental data shows that the error in these quantities is likely to be no more than ±0.1 S. Thus the range of values for the sedimentation ratio is 23.2/8.1–23.4/7.9 i.e. 2.86–2.96. There is no value available for the hydration, but this protein supplies another example of one where the sedimentation ratio itself is high enough to exclude most structures. Examination of fig. 7 of [1] shows that the only structures compatible with the sedimentation ratio are CTV ($P_{1.5}$–P_2); CVV ($P_{1.5}$–$P_{2.5}$): CRT ($O_{1.5}$–O_3). The structures with prolate protomers are virtually indistinguishable and correspond to the pentagonal arrangement, shown in fig. 3, of prolate ellipsoids of axial ratio 1.5–2.5, perhaps twisted slightly with respect to each other. The CRT assembly with oblate protomers of axial ratio about 3 : 1 is also shown in fig. 3.

We may now ask whether it is possible to decide between the alternative structures discussed above by considering what the shape of the protomer might be. For a molecular weight of 160000, and a partial specific volume of 0.729/ml/g [20] a particle with an $S^{\circ}_{20,w}$ of 8.0 S has f/f_0 = 1.32. Oncley's diagram [6] then leads to protomer shapes of $P_{3.5}$ or $O_{3.5}$ if the hydration is assumed to be 0.3 g/g. These are both rather high, especially the prolate one, to be compatible with the structures deduced from the sedimentation ratio, and one might expect that the source of the inconsistency is in either the value used for $\bar{v}$ (calculated from the amino acid composition) or the hydration (assumed), or both. It does not require much modification to these values to reconcile the data. For example, with $\bar{v}$ = 0.729 ml/g an axial ratio of 2.5 : 1 demands a hydration of 0.55 g/g, or alternatively, the same axial ratio may be achieved with an hydration of 0.3 g/g if $\bar{v}$ = 0.75 ml/g.

Thus, consideration of all of the sedimentation data

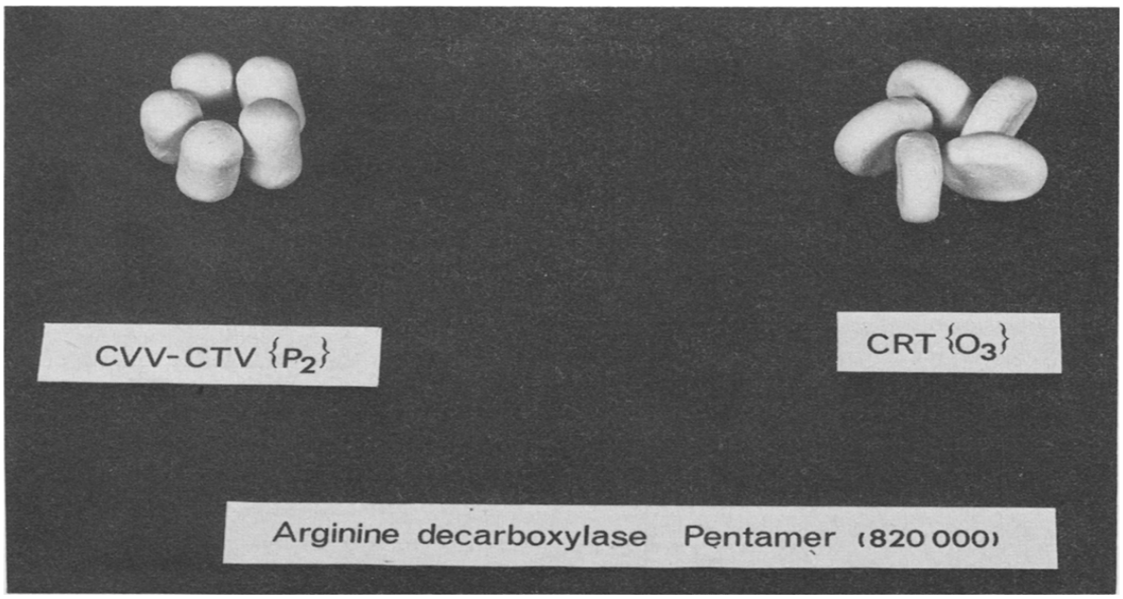

Fig. 3. Alternative structures for arginine decarboxylase. The dimeric protomers in the models all have the same volume. Oblate and prolate protomers are shown as rounded discs or cylinders, respectively, of the appropriate axial ratio. These are effectively the shapes to which the method of calculation refers. Both structures shown are possible on the basis of sedimentation data but the one on the left is favoured since it is also in agreement with electron microscopy.

suggests that the structure of the arginine decarboxylase pentamer is either CRT with oblate protomers of axial ratio about 2–2.5 : 1 or CVV–CTV with prolate protomers of axial ratio about 2–2.5 : 1, a partial specific volume between 0.729 and 0.75 ml/g and a hydration between 0.3 and 0.6 g/g.

There are clear electron micrographs of the arginine decarbocylase pentamer available [21] and on examination of these it may be seen that the structure denoted CVV–CTV in fig. 3 is in excellent agreement with the observed images. These consist of a regular pentagonal arrangement of five apparently identical molecules with circular profiles as viewed in plan. Only a small number of molecules is shown (about 20) and all views of the pentamers seem to be down the five-fold axis. However, the stain distribution argues for a low axial ratio of the protomers, and in the one or two images which may be subunits, the ratio of length to width is 2–2.5 : 1. An axial ratio of about 2 : 1 is of course plausible for the 8S protomer since it is dimeric.

Boeker and Snell [19] report that on reassociation of the 8S species, peaks with sedimentation coefficients of approximately 12 S, 16 S, and 20 S, as well as 23 S are observed. Though no comment on the structures of the corresponding molecules is warranted because of the lack of precise data, it is interesting that the sedimentation ratios with respect to the 8 S species are 1.5, 2.0, 2.5, and 2.9, respectively. As consultation of fig. 8 of [1] shows, these values strongly support the intuitive notion that the series represents the dimer, trimer, tetramer and pentamer of the 158000 molecule.

Finally, it is worth pointing out that the pentameric arginine decarboxylase molecule is an example of the possibility mentioned in [1] of a structure for which the sedimentation ratio fortuitously obeys the $n^{2/3}$ rule, even though it is quite different from that of the protomer.

2.4. Arthropod hemocyanins

A series of oligomeric forms is found variously distributed among the arthropods [22], the most com-

mon aggregate being the hexamer composed of six 75000 molecular weight subunits. Since the molecular weights of the hemocyanin oligomers are in the ratio 1 : 2 : 4 : 8, where the first member of the series is the hexamer [23], it is convenient to refer to the hexamer as the protomer. Then the aggregated forms, usually labelled by their approximate sedimentation coefficients of "24 S", "35 S", and "60 S", can be regarded as the dimer, the tetramer, and the octamer, respectively of the hexameric "16 S" protomer. A possible arrangement of the six subunits in the "16 S" molecule was presented based on the sedimentation ratio treatment [2]. More recent electron microscope and X-ray analysis [24] have suggested an alternative arrangement in which the six protomers are in the form of a trigonal antiprism. It has been shown that a sedimentation coefficient calculated on this basis is in excellent agreement with the experimental value for the *Cherax destructor* hexamer [22]. The difference from the previous analysis lies only in the protomer shape, which, for the later model, is taken to be not far from spherical. The exact arrangement of the subunits within the hexamer is not important for the present purpose, only the overall shape of the hexamer, since we wish to treat it as the protomer for the next association. In fact, both from electron microscopy and the above model, the hexamer is well approximated as a slightly flattened sphere.

In attempting to treat the arthropod hemocyanin oligomers by the sedimentation ratio method it is important to realize that there may be genuine differences in sedimentation coefficients of the same aggregate from different species, as will be seen below. This is most likely because of differences in subunit size since the quaternary architecture seems to be the same throughout the arthropods. Nevertheless it needs to be taken into account here because the entire series of oligomers is not well-represented in any one biological species, and sometimes the lack of a reliable sedimentation coefficient imposes a further limitation on the aim of building up a consistent assembly pattern by applying the sedimentation ratio method.

2.4.1. Dimer

The value of $S^{\circ}_{20,w}$ for the protomer is 17.3 S for *Cherax destructor* hemocyanin and the same value is obtained by extrapolation of a plot of sedimentation coefficient against concentration for combined data for the decapod crustaceans *Pandalus borealis, Astacus fluviatilis, Carcinus moenas,* and *Panulirus vulgaris.* The sedimentation coefficients, obtained from Eriksson–Svedberg and Quensel [25], were plotted on the basis that the hemolymph concentration was the same in all species as that of *Cherax*. For the dimer of *Cherax destructor* $S^{\circ}_{20,w}$ is found to be 25.5 S and this is in good agreement with that of 25.6 S, found by the same method as above, for data from *Nephrops norvegicus, Astacus fluviatilis, Cancer pagurus,* and *Carcinus moenas* [25]. The sedimentation ratio for the decapod crustaceans is thus 25.5/17.3 = 1.47.

It is interesting to compare the values with those found for a member of another arthropod class, namely the spider *Cupiennius salei*, where the two aggregates in question also occur in the hemolymph. The $S^{\circ}_{20,w}$ values for protomer and dimer are 15.9 S and 23.4 S, respectively [26] to give a sedimentation ratio also of 1.47. This implies that although there may be differences in size between oligomers from different arthropod species, the arrangement of subunits is likely to be the same across the phylum. Electron microscope studies have led to the same conclusion [22,23,27]. It may be seen from fig. 3 of [1] that a sedimentation ratio of 1.47 for the dimerization of a slightly oblate protomer predicts a Vr90 structure for the dimer. This is shown in fig. 4 and is in good agreement with 24 S structures observed in the electron microscope [22,23,27].

2.4.2. Tetramer and octamer

The next aggregate, the tetramer, of sedimentation coefficient about " 35 S" is not observed in the hemolymph of decapod crustaceans. It is found in spiders [26], scorpions [28], and the horseshoe crab *Limulus polyphemus* [25], while the octamer (60 S) is found only in the latter. Lack of precise sedimentation coefficients measured under the same conditions for the complete set of oligomers including the tetramer and the octamer introduces a problem in applying the sedimentation ratio method further. Thus, in *Limulus*, the only arthropod for which values for all aggregates are listed, the ranges given are 52.6–59.7 S, 29.6–38.1 S, 23.1–25.8 S, and 14.4–17.6 S [25]. Other values quoted are 62 S or 60 S and 42 S or 40 S, apparently at infinite dilution [29], and 52.8 S and 30.6 S for approximately 0.9% solutions [30]. Like-

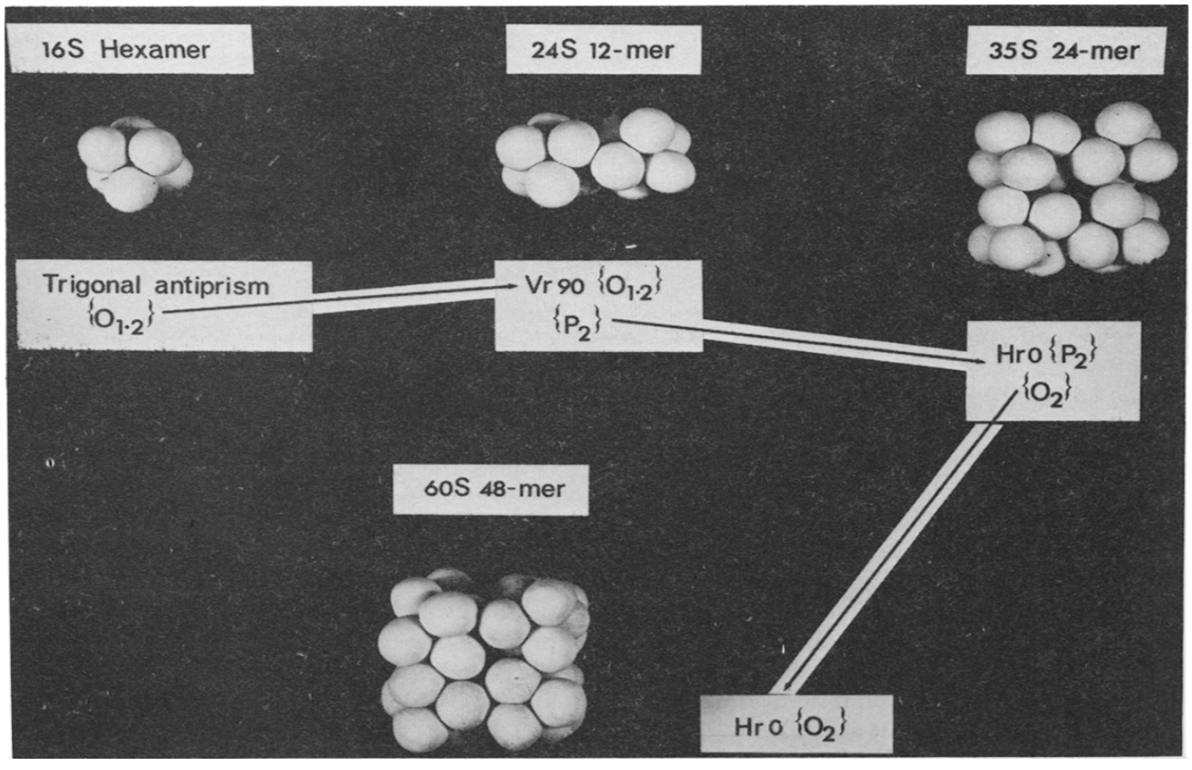

Fig. 4. Structures of arthropod hemocyanin aggregates. The assembly pattern of each oligomer is deduced on the basis of its sedimentation ratio with respect to the preceding one. Thus each oligomer, as indicated by the arrows, is regarded as the protomer in the next association. More details are given in the text.

wise, there do not appear to be precise values available for the sedimentation coefficients of scorpion hemocyanins. On the other hand, where accurate values are quoted, 36.7 S for $S^{\circ}_{20,w}$ of *Dugsiella californica* and *Dugsiella helluo* tetramers, no comparable value is available for the dimer because it is not found in the hemolymphs of these species [26]. In order to proceed then we assume that the hemocyanin oligomers from spiders, scorpions and horseshoe crabs may be treated together. There are good grounds for the assumption. The subunits from these species show considerable antigenic identities and also an ability to form hybrid oligomers [23].

Now if the $S^{\circ}_{20,w}$ of 36.7 S for the *Dugsiella* tetramer and that of 23.4 S for the *Cupiennius* dimer [26] are combined, a value for the sedimentation ratio of 1.57 results. It has already been seen that the dimer, as a Vr90 structure of almost spherical protomers, is essentially a P_2 protomer for the next association (fig. 4) and this, together with the sedimentation ratio shows that the tetramer may be described as Hr0–P_2 (fig. 3 [1]). The resulting structure is in excellent agreement with the appearance of images of this oligomer seen in electron micrographs of spider [27], scorpion [23] and *Limulus* tetramers [23].

A definitive $S^{\circ}_{20,w}$ value for the *Limulus* octamer is not available, however, it may certainly be taken to be formed by aggregation of two of the tetramers discussed above. Inspection of fig. 4 shows that these, which are planar arrangements for almost spherical hexameric protomers, can be regarded as O_2 protomers. Fig. 3 of [1] shows that the most likely mode of assembly for two such protomers to form the octamer is Hr0 with a sedimentation ratio of 1.635. This leads to a value of 60.0 S for the octamer if the tetramer sedimentation coefficient is taken as 36.7 S, the value for *Dugsiella*. The Hr0 structure formed by the aggregation of two tetramers is shown in fig. 4 and is

in good agreement with the conclusions of electron microscope analysis of the *Limulus* octamer [23].

The sedimentation ratio analysis suggests that the dimer, the tetramer, and the octamer of arthropod hemocyanins have the assembly patterns illustrated in fig. 4, and these are in agreement with analyses based on the appearance of negatively stained molecules in the electron microscope. It seems that these aggregates, and the arrangements of the molecules within them, account for most of the arthropod hemocyanin oligomers. Two possible exceptions should be mentioned. The first is an aggregate of sedimentation coefficient about 30 S observed in *Cherax* hemocyanin [22]. Such a molecule may also occur in the hemolymph of *Homarus vulgaris* [31]. It is possible that these are trimers of the "16 S" hexamer since the sedimentation ratio with respect to the hexamer appears to be in the appropriate range (fig. 8, [1]). The second interesting example is provided by the tetramer of *Callianassa californiensis* hemocyanin. In their original report Roxby et al. [32] thought the tetramer was likely, on the basis of its sedimentation coefficient, to consist of a planar arrangement of four 16 S molecules. Later they reported that this assignment may have been in error since the appearance of the molecule in the electron microscope is tetrahedral [33]. This molecule thus presents a problem because the ratio of the sedimentation coefficients ($S^{\circ}_{20,w}$ values), being 2.27 (38.8/17.1), is just what would be expected for the cyclic arrangement of four near spherical protomers (fig. 6 [1]), which the sedimentation analysis above, and the electron microscopy, shows is typical of other arthropod hemocyanins. One possibility is that the tetrahedral molecules observed in the electron microscope are not hemocyanin but a non-respiratory protein of the type observed by Markl et al. [34] in spider hemolymph; this also had a tetrahedral appearance.

3. Discussion

In the work described in the present two papers, and in the previous one [2], we have tried to present a general method which may easily be applied to experimental sedimentation data and which covers most of the possibilities of protomer shape and mode of assembly likely to be encountered with oligomeric proteins. Despite limitations it does, we believe, provide a more systematic and realistic approach to the analysis of sedimentation data than that restricted to assemblies of spherical protomers. It is of course far from exhaustive; other modes of assembly could be considered, including spatially non-equivalent cases. In particular it should be mentioned that Kirkwood's equations provide the means to calculate sedimentation coefficients for more elaborate structures with more refined protomer shapes if there is evidence from other sources which warrants such an approach. This kind of application is illustrated by the work of Bloomfield et al. [35] with molluscan hemocyanins and viruses, Filson and Bloomfield [36] with polymers and Uedaira and Uedaira [37] with small sugar molecules.

The results presented above indicate that the use of the calculated sedimentation ratios in the manner discussed may be expected to give reasonably good results. Thus, in the cases considered where it was possible to compare structures deduced on the basis of sedimentation ratios with those from other methods, it was found that those derived on the former basis at least included the "correct" structure; often there are not many alternative possibilities. With reference to potential limitations of the method, mentioned in [1], it appears that the most serious in practice are lack of precise sedimentation coefficients measured under the same conditions for protomer and oligomer, and ambiguity in the choice of protomer shape. In an experimental study of a particular protein the former could usually be overcome readily, while, if it were considered to be justified, more information about the protomer shape could also be obtained by applying different experimental techniques [1,6]. In this connection one needs to be clear about the level of resolution being aimed at in the description of oligomer geometry. It is apparent that, although the method can be surprisingly useful, especially in conjunction with results from other techniques, what can be achieved from comparisons of sedimentation coefficients alone is necessarily limited. Thus the additional work involved in achieving finer resolution might be better invested in a more appropriate technique.

We hope that the level at which the sedimentation ratio approach can be useful has been illustrated and that a degree of versatility has also been demonstrated; not just in the way the calculated sedimentation ratios

may be applied to the nominal oligomer types discussed, but also to larger aggregates by suitably approximating associated structures as protomers.

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